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Industrial and Environmental Biotechnology

Achievements, Prospects, and Perceptions



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Foreword

Biotechnology applications in agriculture are well known, but its industrial applications are now beginning to emerge. The so-called “White Biotechnology” points to an emerging field in biotechnology with immense potentials and significant impact on human socio-economic development. The chemical industry would be transformed through biotechnology innovations and policy makers, industrialists, researchers and consumers will all have to embrace and adjust themselves to the challenges of biotechnology.

As these promises open up new opportunities for development, they also pose questions on old concerns such as the development gap between the developed and developing countries, poverty alleviation, and sustainable development in general. The impact of White Biotechnology on the ability of the international community in addressing common concerns deserve attention, for example its impact on biodiversity and intellectual property rights is largely unknown.

This report is part of a series of publications by UNU-IAS on biotechnology. It is a consolidation of the knowledge and promises in White Biotechnology. It cites progress in various areas such as industrial enzyme development, progress in bioplastics production and biofuels, applications in the paper industry, biomining and environmental applications. These advances constitute a new front for society to interact with science but they will also point to future policy challenges for development and think-tank organizations such as UNU-IAS.

Being an institute for advanced studies, among the objectives of UNU-IAS is to promote dialogues between science and society to inform policy-making. I hope this report would generate interest and new ideas among policy makers, professionals, scientists and other groups who are concerned and hopeful of the promise and potential of biotechnology in human welfare and development.

A.H. Zakri
Director, UNU-IAS

Executive Summary

'White' biotechnology or industrial and environmental biotechnology is a broad and expanding field that includes making enzymes with a variety of industrial uses that include the manufacture of bioplastics and biofuels and using micro-organisms and plants for the treatment of wastes and abatement of pollution, a process known as bioremediation. It may seem to be occupying a small niche now, but its products or processes will become as ubiquitous as those of the chemical industry today because of the benefits they pose to the environment and the premium attached to this environment-friendliness for the companies that undertake them. Bacterial bioleaching, i.e. using specific bacteria to extract metals from ores or mine wastes sprayed with water, is a growing sector of the mining industry and several developing countries are already playing a key role in this area. Other research efforts are scanning the microbial diversity of various environments (e.g. the oceans and seas) and deciphering their genetic information aimed at isolating micro-organisms that could be used in the manufacture of drugs, enzymes and a wide range of bioactive compounds, as well as in bioremediation processes. As for materials and fuels that are not derived from petrochemical processes, researches are also underway for processes to improve and enhance the bioremediation of water, soils and ecosystems at large, and minimize the use of fossil-fuel energy. All these forms of 'white' biotechnology are poised to increase the field's positive social acceptance.

1 Definition and policies

The so-called 'white' biotechnology is a broad and expanding field that makes use of new enzymes for a variety of industrial uses, embraces the manufacture of non-oil-based and biodegradable bioplastics and biofuels, as well as artificial fibres and encompasses many treatments of wastes and abatement of pollution, using micro-organisms and plants, known as bioremediation. White biotechnology is part of the contribution of applied science to a healthier environment and to sustainable development (The Economist, 2003).

'White' biotechnology or industrial and environmental biotechnology may now only seem to occupy a small niche but because of its enormous promise and potential, it may become as ubiquitous as those of the chemical industry today. Some analysts have compared the current status of biotechnology to that of chemistry in the 1870s, when chemists were applying their new knowledge to a limited range of applications (e.g. dyes). Now, industrial chemistry is everywhere, from agrifood and pharmaceutical industries to fuel production to textile, fertilizer, paper industries, etc. (The Economist, 2003).

From the policy viewpoint, after the pioneering efforts by the US National Academy of Sciences at the end of the 1980s, the future-oriented studies initiated in 1996 and the first report by the Organisation for Economic Cooperation and Development (OECD) in 1999 on 'white' biotechnology, a decree signed by the US President William Clinton on 12 August 1999 on 'Developing and promoting bio-based products and bio-energy' stated that between 2000 and 2010 the consumption of biotechnology-derived products and fuels should be trebled. In June 2000, the Biomass Research and Development Act and the Sustainable Fuels and Chemicals Act, voted by the US Congress, allocated hundreds of million dollars to research projects jointly carried out by universities and industry; they also created the Biomass R&D Board, in charge of coordinating the action of federal administrations (commerce, energy, agriculture, etc.) [Reverchon, 2002].

Later on, US President George W. Bush strengthened this framework in the Farm Bill. Behind this decision was the need to find outlets for agricultural surpluses, to ensure national security by decreasing the dependence on fossil fuels, and to poise US farmers to become the future suppliers of feedstock to the industry. The US industry could also look at this as a way to boost their competitive edge over European corporations.

Meanwhile, in Europe, the Biowise Programme of the British government had surveyed 70,000 industrial installations, which could reduce costs and decrease pollution through biotechnology. The government wanted to convince these industries to invest in this area. BASF AG in Germany, the Dutch chemicals company DSM and most of Scandinavian paper-manufacturers were already doing so. The European Union's heads of state and government, at the 2001 Göteborg Summit, stressed the need for sustainable development in Europe, and in March 2002 in Barcelona, they underlined the key role of biotechnology to achieve it. On 14 July 2002, the European Commissioner for Research announced that €3.42 billion were to be devoted to the

programme 'Clean technologies' between 2002 and 2006. Earlier on, on 4 July 2002, the European Parliament voted a directive on biofuels that indicated a target of 5.75 per cent for the proportion of biofuels consumed for transport by 2010, a great increase from the 0.3 per cent in 2002 (Reverchon, 2002).

In its long-awaited environmental plan (ETAP), released at the end of January 2004, the European Commission identified 'white' biotechnology as an "important technology" with a bright potential for environmental benefits. The action plan foresaw opening up new financial resources from the LIFE Programme or structural funds to finance pilot and demonstration projects. Additionally the Commission aimed to put in place some solid science to improve the market acceptance of the innovation technology so they also envisioned the creation and funding of expert networks that should validate the biotechnological processes. 'ETAP was designed to deliver environmental benefits and give a boost to innovation', stated Steen Riisgaard, chair of the Industrial Biotechnology Board at the European Bio-industries Association (EuropaBio).

2 Enzymes

Purifying and selling bacterial enzymes for use in cheese making, food manufacturing, cotton weaving, washing powders, etc., has existed for decades, but in 1988 the Danish company Novozymes produced the first transgenic enzyme, a fat-digester for detergents. The company supplies, among others, enzymes for the scouring process, which consists of removing the brown, non-cellulose parts of cotton that in the conventional process involves the use of relatively harsh chemical solutions. Thanks to advanced genetic techniques, multiple enzyme variants can be created at high speed, which are then screened to fit the desired applications. Novozymes has become the world's biggest enzyme manufacturer, and also renowned for its transparency procedures, for involving the consumers in the discussion of its activity report, and its commitment to a healthier environment and to sustainable development.

Genencor, created in 1982 as a partnership between the biotechnology company Genentech, Inc., and the chemical firm Corning, had an annual turnover of \$325 million in 2001 and a portfolio of 3,400 patents and licenses. Its main income is from the sales of enzymes, which are discovered, tested and standardized in Genencor's laboratories at Palo Alto (California) and Leiden (Netherlands). At the Leiden laboratory, established in 1995 in the BioScience Park, some 25 researchers are trying to improve the performance of enzymes developed for Procter & Gamble, Unilever and other detergent manufacturers (by 2002, the detergent market value was estimated at \$400-\$500 million a year). Genencor is famous for having isolated an enzyme that gives the stone-wash colour to jeans, thereby replacing the process, which consisted of washing the jeans in machines with volcanic stones (Reverchon, 2002).

Genencor's enzymes are being massively produced in bioreactors located in the USA, United Kingdom, Belgium, Finland and China. In addition to the enzymes mixed with detergents, the company had signed a \$17-million contract over three years to work on enzymes involved in the production of biofuels. Genencor is also involved in vitamin-C production with Eastman Chemical, biopolyester manufacture with E.I. DuPont de Nemours & Co., Inc. (DuPont), and sugar with soft drink companies (Reverchon, 2002).

Genencor is also trying to develop enzymes for manufacturing hypoallergenic proteins, which would penetrate the cosmetics and body-hygiene industry – a \$1 billion market. The company is interested in developing biosilicones in association with Dow Corning which brought in \$35 million. Genencor is looking to two to three years to extend the market it would have captured and create new ones in four to ten years. Meanwhile, it will patent technological innovations while waiting for a market that may emerge in the longer term (Reverchon, 2002).

Sigma-Aldrich Fine Chemicals, a division of Sigma-Aldrich Corporation, well known for its expertise in protein production and processing, agreed with ProdiGene, Inc., a Texas-based biotechnology company, to manufacture and distribute TrypZean, an animal-free recombinant trypsin, produced with ProdiGene, Inc.'s proprietary transgenic plant system. Trypsin is an enzyme normally produced in the pancreas of animals

that breaks down proteins as part of the digestion process. It is also a critical enzyme in the bioprocessing of proteins, such as insulin, where it is used to help cleave the protein into its active form. Trypsin is widely used in cell-culture applications for research on, and production of, recombinant proteins for clinical uses. Another application is its use in the wound-care markets as an oral treatment of inflammatory oedema, haematoma and pain associated with a wide variety of internal and external wounds. The enzyme also has uses in food processing and in the making of infant formulas. Concerns about the use of animal- and human-derived proteins in the manufacture of biologicals make ProdiGene, Inc.'s expertise in the area of plant-produced recombinant proteins an attractive alternative. The proprietary technology offers an animal-free source of proteins as well as the capacity to produce large volumes that can be easily scaled-up or down (The Economist, 2003).

Extremophiles, i.e. micro-organisms living in very harsh natural conditions, have attracted the attention of scientists who are collecting them from volcanic areas, hot springs, deep-sea vents, soda lakes, arctic rocks and industrial effluent outlets. This practice called "bioprospecting" is followed by the study of metabolism of the micro-organisms and the isolation of enzymes and other proteins with extraordinary properties, such as tolerance for heat, cold or salt. These properties make them suitable for use in the manufacturing of washing powders, in textile industry and bioremediation processes. Japanese companies are very active in this bioprospecting and research area, as well as Costa Rica whose great biological diversity is not only due to the preservation of its rainforests but also to the existence of numerous extreme environments (mainly volcanic areas). Other firms like Applied Molecular Evolution, Genencor and Maxygen, interested in the search for drugs, are attracted by the extremophiles, and their peculiar metabolism and evolution. See also Sasson, A., 2000.

India too is a player in biotechnology with the bulk of India's industrial biotechnology sector focuses on producing enzymes for the textile and paper industries. The sector was the second-largest exporter (\$37 million in 2002-2003) to other Asian and European countries. In 2002-2003, this sector produced revenues of \$72 million and was rapidly growing (Grace, 2004).

The example of vitamin-B₂ synthesis illustrates how a new biotechnology process can benefit both environment and economics. This vitamin is generally produced using a complex eight-step chemical process. BASF AG's new process reduces it to a one-step process involving fermentation whereby the raw material is fed to a mould, which transforms it into the finished product, recovered as yellow crystals directly from the fermentation broth. The biotechnological process reduces overall costs by up to 40 per cent and the environmental impact by 40 per cent (CO₂ emissions are reduced by 30 per cent, resource consumption by 60 per cent and waste by 95 per cent). The synthetic pathway to the antibiotic cephalixin has also evolved from a multistep chemical process to a mild bio-transformation, based on a fermented intermediate linked enzymatically with a side chain to the final end product. The biotechnological process uses less energy and input chemicals, is water-based and generates less waste.

3 Bioplastics

Henry Ford, a great American inventor, was fascinated with soybeans. During the Depression, he spent many hours in a laboratory near Detroit trying to transform the beans into cheap plastic, so that more Americans could afford his Model T car. By 1941, Ford eventually unveiled a hand-made car with a plastic body made completely from plants but unfortunately, he never figured out how to produce soybean-based plastic that could compete on price with oil-derived plastics, still the dominant type of industrial polymers (The Economist, 2004a).

Currently, the two most advanced plastics projects are those of E.I. DuPont de Nemours & Co., Inc., one of the world's biggest chemical corporations (which invented nylon), and of Cargill-Dow Chemical Company, a joint venture between an agricultural and a chemical firm. Established in 1865 and headquartered in Minnetonka, Minnesota, Cargill, Inc., described itself in its 1997 financial report as 'an international marketer, processor and distributor of agricultural, food and industrial products with some 79,000 employees in more than 1,000 locations in 72 countries and with business activities in 100 more'. Cargill, Inc.'s global revenues peaked at \$56 billion in 1996 and 1997, dropping somewhat to \$49.4 billion in 2001. Dow Chemical Company is headquartered in Midland, Michigan. The joint venture was spun off from Cargill, Inc., in 1997 and employed about 230 people (The Economist, 2003).

DuPont's process, developed in collaboration with Genencor, consisted of assembling in a single bacterium biochemical pathways from three different micro-organisms. The raw material for the process is glucose syrup made from maize starch, which is converted into 1,3-propanediol, used to manufacture a polyester called Sorona. The latter is not entirely a biopolymer, but a copolymer, made of 1,3-propanediol and terephthalate, which still has to be made from oil. Cargill-Dow Chemical Company's product, Ingeo, is made out of lactic acid, which in turn is produced from glucose derived from non-transgenic maize starch. Polymerization of lactic acid, through traditional techniques leads to poly-lactic acid (PLA), i.e. Ingeo or NatureWorks PLA. In January 2002, Cargill-Dow Chemical Company opened a \$300-million plant at Blair (Nebraska) that could manufacture 140,000 tons of Ingeo, biomaterials to be mainly used in packaging and bedding. This plant was the first to produce biomaterials on a commercial scale. As Ingeo was more expensive than petrochemical plastics, Cargill-Dow Chemical Company intended to introduce it in the market of environment-friendly goods, which were sold at a higher price. This friendliness is due to the fact that no (or little) fossil fuels are used to manufacture the biopolymers and that they are biodegradable so they do not contribute to global warming or to pollution. Coca-Cola Co. is now using Ingeo to make soft-drink cups, McDonalds for salad containers, and Pacific Coast Feather Co. to fill pillows and comforters. If the production cost of bioplastics could be halved, the amount produced in 2010 could be trebled (Reverchon, 2002).

Small firms are also interested in the manufacture of biopolymers. Metabolix, a company based in Cambridge, Massachusetts, is working on the production of monomers by living organisms and on their polymerization by the

same organisms. James Barber and Oliver Peoples, the founders of Metabolix, working in the early 1990s at the nearby Whitehead Institute, realized that some bacteria could store polyhydroxyalkanoate up to 80 per cent of their weight. Polyhydroxyalkanoates (PHAs) are produced by either non-recombinant bacteria (e.g. *Ralstonia eutropha*) or recombinant ones (e.g. *Escherichia coli* to which genes of *R. eutropha* have been transferred). The production cost of bacterially produced PHA was between \$3 and \$5 per kg, while that of propylene was less than \$1 per kg. There is therefore a need to decrease the production cost and also increase productivity (Dufour, 1999). Metabolix planned to start commercial production of PHA by the end of 2003, at a price comparable with that of the more expensive polyesters. The company hoped that biodegradability and tougher regulation on the disposal of synthetic plastics into the environment would give a competitive edge to its products over the tried and trusted chemical materials (The Economist, 2003).

In 1989, researchers of the French Development Research Institute (IRD) discovered in palm wine the bacterium *Bacillus thermoamylovorans*, which is able to produce lactic acid from sugar-rich wastes. In cooperation with a subsidiary of France's third-biggest sugar producer (Erstein), Epicure, the first production trials were carried out in April 2000 on three kinds of juices which demonstrated the efficiency of the process and led to high yields of lactic acid (100g/litre). The bacterium grows at a temperature between 47°C and 58°C – which avoids the contamination by other micro-organisms. The Research Centre for Artificial Biopolymers (CRBA), in Montpellier, south-east of France, has also been developing a polymerization process of lactic acid, in order to produce bioplastics on a commercial scale.

All companies working in this field are trying to bring down costs. Metabolix, for instance, hopes to switch from producing plastics from bacteria (which need to be fed) to manufacturing them in plants (through photosynthesis). The process is being scaled up from successful laboratory trials. It is also foreseen to produce plastics in transgenic plant species.

In 1992, French researchers were able to transfer two genes of *R. eutropha* into *Arabidopsis*, which could produce 0.1 per cent of its biomass as PHA. In 1994, this proportion was increased up to 14 per cent after the transfer of a third gene and a better expression of alien genes. This yield was considered satisfactory, but the polymer had poor physical properties. In 1998, small quantities of a good quality PHA, called MCL-PHA, could be derived from a transgenic plant; this plastic resembled elastomeres, rubber or glue and since the market for elastomere-like materials was less important than that of plastics used for packaging, large-scale production was considered less crucial (Dufour, 1999).

Clint Chapple a biochemist of Purdue University, and Knut Meyer of E.I. DuPont de Nemours and Co., Inc., have cloned a gene of *Arabidopsis* which could influence the synthesis of plastics in plants without harming their structure. The scientists stated they would be able to produce plastics that could serve for the manufacture of heart valves, in the building of aeroplanes and other sophisticated uses.

Plants could produce the monomeric units of the plastic polymers, as some monomers are difficult to elaborate from oil. C. Chapple's work consisted of trying to stabilize some monomers and to produce them in high concentrations in plants. The viability of the whole process depended on the storage of sufficient amounts of the plant-made plastics in the cell vacuoles from which they could be extracted (The Economist, 2003). The gene was eventually patented in favor of the University and the US corporation.

The results published by Steven Slater and his collaborators in the October 1999 issue of Nature Biotechnology also opened new prospects for the large-scale production of a very good quality PHA from the leaves of transgenic *Arabidopsis* as well as from the seeds of transgenic oilseed rape. The US researchers had transferred four bacterial genes to the plants that could be expressed in two distinct metabolic pathways. However, both transgenic *Arabidopsis* and oilseed rape produced only 2.5 per cent of their biomass as plastic, which implied that more genetic transformations would be necessary to obtain commercially exploitable results. The extraction process was also an important element of a profitable production of plastics by transgenic crops (Dufour, 1999).

There is also a research consortium comprised of Malaysian scientists and scientists of the Massachusetts Institute of Technology that has been working on the development of transgenic oil-palms that produced a bioplastic in their leaves. The genes transferred to the tree were those of *Ralstonia eutropha*, which control the synthesis of polyhydroxyalkanoate and polyhydroxybutyrate – the precursors of the bioplastic. The latter was to be recovered from dried leaves, currently used as feed or for fibre production; it will not be present in the fruit bunches, used to extract palm oil. By the end of 2003, trials were expected to be carried out to test the production capacity of transgenic plants. See also Sasson (2000).

4 Feedstocks and biofuels

Another promising biotech idea is to use the whole plant as a chemical feedstock. Glucose syrup is a refined product, made out of maize starch, i.e. maize grains. The latter costs about \$80 a ton; that is cheaper than oil, weight for weight, but this could be improved if the plant waste is also used, as it fetches only about \$30 a ton for silage. But it consists mainly of cellulose, also a polymer of glucose, but more difficult to break down; therefore, the search began for efficient cellulases. Verdia, Maxygen's plant-biotechnology subsidiary, is trying to develop a cellulase that the plant would make in its own cell walls, while preventing the enzyme to digest the living plant. If research and development were successful, limitless supply of glucose from the degradation of cellulose would follow, as well as all kinds of bioplastics (The Economist, 2003).

4.1 Ethanol Production

The OECD (Organisation for Economic Cooperation and Development) and a handful of big corporations already acknowledge that the fossil-fuel era will come to an end and that industry has to prepare for it. Governments are helping drive the shift to alternatives through subsidies and regulations. The USA, for instance, published draft rules to encourage federal purchasing of bio-based industrial products in 11 categories, from lubricants to fibres, plastics and paints. It also subsidizes making ethanol from maize kernels. The EU targets under the Kyoto Protocol, have their eye on some 9.3 million tons of ethanol that should be produced annually in Europe by 2010. The biofuels target for 2005 is 2 per cent of vehicle fuel, rising to 5.75 per cent by 2010 (The Economist, 2004a).

Biofuel production is part of 'white' biotechnology. Ethanol, a biofuel, is produced from the fermentation of cane sugar. In Brazil, they have mastered the technology as well as that of building motor engines that use this rather corrosive fuel. The decision was made to decrease the amount of the oil bill and to seek energy independence. Ethanol served to power the first car of Henry Ford, while nowadays in Brazil an important proportion of the car fleet uses a mixture of gasoline and 20 per cent ethanol. Even in the USA, nearly a tenth of all motor fuel sold is a blend of 90 per cent petrol and 10 per cent ethanol.

The American market for bioethanol is 8 billion litres a year. At Genencor, some reckon it could be as high as 75 billion litres a year by 2020. That would be enough to replace two-thirds of US current petrol production. Royal Dutch Shell and Iogen, a Canadian firm, were among those building an ethanol refinery that converts 'residual biomass' – leftover plant matter usually thought of as waste. Thanks to the creation of more efficient enzymes that cut fermentation costs, advances in transgenic maize that have improved yields, and government subsidies, 80 US ethanol refineries produced a record 11 billion litres in 2004, or less than 2 per cent of all motor fuel used. This figure is already very close to the US Congress target of 19 billion litres of maize-derived ethanol (2.5 per cent of all motor fuel used) set by year 2012.

Canada intended to quadruplicate its ethanol production, up to 1 billion litres, between 2000 and 2005. Brazil – the

world's first-biggest producer of ethanol – produced 15 billion litres annually (2003). In 2003, Europe produced 700,000 tons of biofuels, half of it in France.

While the production, transport and consumption of gasoline generate 11.8 kg of CO₂ per gallon (3.8 litres), in the case of ethanol 7 kg to 10 kg of CO₂ are generated if conventional production processes are used, but only 0.06 kg if one relies on bioprocesses (Reverchon, 2002). Although motor fuels mixed with ethanol could cut hydrocarbon and carbon monoxide emissions, they also produced aldehydes and peroxyacyl nitrates, which could form brown smog. Ethanol producers have previously fallen foul of the US Environmental Protection Agency (EPA) for emitting volatile organic compounds such as formaldehyde and acetic acid, and methanol. Techniques for efficiently disposing of large volumes of these chemicals must be developed and strictly applied (The Economist, 2004).

4.2 Biofuel production in the European Union

In the European Union, in 2003, two directives have set the following targets for biofuel content in the currently used fuels: 2 per cent in 2005 and 5.75 per cent in 2010. The biofuels produced in the European Union's member countries, particularly in France are the following:

- methylester of vegetable oil or biodiesel (diester), produced from groundnut, oilseed rape or sunflower seeds; soybeans and palm oil can also be used; the extracted oil is transformed into methylester, incorporated to gasoil by the oil-industries;
- ethanol, produced from sugar-cane, sugar-beet, wheat or maize; in Europe, it is transformed into ethertertibutylether (ETBE) – a mixture of isobutylene, a by-product of the oil industry, and ethanol – before being mixed with petrol; such mixture, considered technically justified by the oil industry, is questioned by ethanol-producers who recommend the direct incorporation of ethanol into petrol as it is done in Brazil and the USA (Dupont, 2004).

The maximum biofuel content of current fuels is 5 per cent; it can be increased to 30 per cent in the case of collective transport means or of vehicles under public administration. In France, the average rate of incorporation of biofuels is only 1 per cent (Dupont, 2004).

The volume of production of biofuels is regulated in France through the allocation of tax exemptions, which amounted to €350 per cubic meter (m³) for diester for a production quota of 387,000 tons. While 300,000 hectares were planted with oilseed rape in 2004 to produce biofuels, specialized professionals estimated that 800,000 ha could be devoted to this crop for the same purposes. With respect to ethanol, France was producing 1.3 million hectolitres on 25,000 hectares of sugar-producing crops; with the rate of tax exemption was €380 per m³ (Dupont, 2004).

To reach the target of 5.75 per cent of biofuel content in the currently used fuels as set by the European Commission, the

following surfaces could be cultivated with wheat (309,000 ha, i.e. 7 per cent of the whole surface) and sugar-beet (62,000 ha, i.e. 14 per cent of the whole acreage), which corresponded to 3.9 per cent of the available area. The European Commission supports the cultivation of energy-producing crops at the maximum rate of €45 per hectare (Dupont, 2004).

In France, a plant built up in 1993 in the heart of the harbour and industrial zone of the city of Rouen, produces 350,000 tons of vegetable oil annually from 800,000 tons of oilseeds supplied by farms in the north-west of France. Two-thirds of the volumes of oil thus produced were transformed into biofuels in 2004. The latter were not substitutes for current fuels, but complements that were mixed with petrol or gasoline. Such a factory bears witness to the period when France was pioneering the production of biofuels. After the reform of the Common Agricultural Policy (CAP) in 1992, which introduced the fallow scheme, farmers have been authorized to grow energy-producing crops. Large-scale agricultural zones, such as Beauce and Picardie, seized this opportunity with the government's assistance but they soon reached a plateau in their production capacity. The forthcoming reform of the CAP in 2006 was expected to oblige farmers to find new outlets for their biofuel products (Dupont, 2004).

A study carried out under the aegis of the French Agency for Environment and Mastering of Energy (ADEME) and of the Directorate of Energy Resources of the Ministry of Industry, published in September 2002, emphasized that the energy balance of biofuels was very positive: they produced more energy than they consumed in their production, in contrast with gasoil and gasoline. The development of biofuels could lead to diversifying energy supply in France, but the main obstacle to this development is their cost, which is two to three times higher than that of oil products and derivatives. Therefore, in the absence of fiscal aid, the consumer will have to pay more for fuel. Another constraint relates to the balance between the market of petrol (gasoline) and gasoil: the European Union was importing 22 million tons of gasoil and exporting 20 million tons of gasoline, mainly to the USA. Such a situation is explained by the strong increase in the number of diesel engines, nowadays found in one car out of three in Europe. While the demand for gasoil was increasing by 2.5 per cent annually, gasoline consumption was decreasing by 1.5 per cent (Lauer, 2004).

Car manufacturers are also worried about the impact of ethanol on modern engines, compared to other mixtures like diester or biodiesel, which behaves like ordinary fuel. According to ADEME, fuel mixtures with 5.75 per cent ethanol, as recommended by the European directive, would generate problems of when it breaks down and at certain temperatures the formation of vapour bubbles that could disturb the injection systems. However, in Brazil, motor engines work with fuel mixtures containing up to 22 per cent ethanol, but engines with injection devices are not very common in Brazil (Lauer, 2004).

Biofuels could help car manufacturers meet their commitment on reducing CO₂ emissions. It was estimated that the European directive on the use of biofuels would

reduce CO₂ emissions by 3.5 per cent; this reduction, although modest, was significant when the decrease in the consumption of motor-cars had reached incompressible limits, and when new devices such as hydrogen-powered cars were not expected before 2024 (Lauer, 2004).

While it is true that biofuels cost more than fuels derived from fossil energy, the real cost of the latter does not integrate the heavy costs of shore and sea contamination by oil and oil-tanker wreckage, nor those of conflicts involving multiple stakeholders generated by oil exploitation. Biofuels have positive effects on employment, tax recovery and supply reliability. The impact on local employment has been evaluated at 6 to 10 jobs created for every thousand tons produced. In fact, on 28 April 2004, the French Economic and Social Council adopted a report on the 'non-food outlets of agricultural commodities' that was very favourable to biofuels. The report stated that 'we should not wait for oil prices to reach \$50 per barrel (the level that would make biofuels competitive) in order to set up substitution processes'. In addition, the rapporteur found it worrisome to see France 'being outpaced by Spain in the case of ethanol production and by Germany in the case of biodiesel', and he criticized the 'taxation disparities' in the European Union as these may hamper the development of biofuels (Dupont, 2004).

The strong increase in biofuel production and the demand for their use would mean an increase in the area cultivated with energy-producing crops. Taking into account the currently available cropping areas as well as crop rotation, biofuel potential is limited to 4.5 per cent of fuel use, lower than the 5.75 per cent recommended by the European directive. The solution may be to produce biofuels from the biomass, i.e. produce larger volumes with an energetic yield two to three times higher than ethanol. The main problem remains the magnitude of investments needed to put in place such an industrial process. The SunFuel programme, funded by Volkswagen, Daimler Chrysler and Renault, has set up pilot units, but generalization of these valuable experiments would certainly need a few more years (Lauer, 2004).

One way to compensate Europe's deficit in biofuels may be to import them from Brazil, especially ethanol, at lower cost. However, trade negotiations with Mercosur's member countries (Argentina, Brazil, Paraguay and Uruguay) in that respect caused panic among farmers in France, who considered that imports would kill the French endeavour to produce such biofuels. At the same time, farmers' associations underlined that biofuels should not hinder food-crop production, considered the primary task of agriculture, and that the foreseen intensification needed to increase yields of energy-producing crops was not in conformity with the goal of a 'rational' agriculture, using less fertilizers and pesticides, and relying more on crop rotation. This opinion was shared by Greenpeace (Dupont, 2004).

4.2 The Paper Industry

The paper industry's basic raw materials are trees. Separating cellulose from lignin in wood is costly and

uses non-environment-friendly chemical processes. Researchers at the State University of North Carolina have bred aspens with only half the lignin of ordinary ones and, it turned out, they had the additional advantage of growing faster. However, the commercial use of transgenic trees is at least ten years off but is on its way (The Economist, 2004 b).

The same is the case with starch, which papermakers use to bind the pulp fibres together and to 'size' the surface, so one can print on it. In Europe and North America, the starch is often extracted from potatoes. But potatoes produce two polymers making up starch: amylopectin, which papermakers like, and amylose, which they dislike. In the 1990s, the world leader in potato starch, AVEBE, a Dutch cooperative, developed a transgenic potato containing more amylopectin, less amylose, but was thwarted by the European Commission, which forbade its marketing. AVEBE grew a new variety, though it would be years before it could reach the market. Through a Swedish subsidiary, BASF AG also created a high-amylopectin transgenic potato; the Swedish authorities gave permission for its large-scale planting in April 2004. The European Commission's consent was required for the growing of this potato variety elsewhere in Europe (The Economist, 2004 b).

But potatoes need not be the only source of genetically engineered starch. The world was producing 190 million tons a year (2003) of cassava, nearly all for food or animal feed. But its starch too can be used for making paper, and in Thailand a little is already used for that purpose. But that could soon become a lot: Thailand is growing enough cassava to be the only significant exporter, and recently decided to allow commercial cultivation of transgenic crops (The Economist, 2004b).

5 The future of industrial biotechnology

In the USA in 2001, the Biomass R&D Board had surveyed 134 industrial sites that were manufacturing bioproducts and found 440 of which produced bioenergy. According to a report published in 2001 by McKinsey & Company, this consultancy reckoned that about 5 per cent of global chemical sales were derived in part from industrial biotechnology. That figure could reach 20 per cent by 2010, if chemical firms, whose polymer innovation had stalled in recent decades, became convinced by the economics of bio-based products. It means that industrial biotechnology will be competing in a market worth \$280 billion, of which McKinsey assumed that it might capture about \$160 billion. The report by McKinsey & Company showed that the greatest impact would be on the fine chemicals segment, where up to 60 per cent of products could be derived from biotechnological processes by 2010. Enzymes and fermentations were already used in the production of flavours and fragrances, while other markets would still be dominated by conventional chemistry through 2010 and beyond. The first applications in the largest volume segments – polymers and bulk chemicals – have already been commercialized. However, in these largely cost-driven segments, a number of technological advances and policy measures would determine the ultimate uptake of industrial biotechnology. McKinsey's estimates showed that the chemical industry alone could generate additional added value up of to €11 billion or €22 billion per annum by 2010, depending whether uptake was fast or slow. Two inputs would contribute to this. One was lower costs for raw materials and processing, combined with smaller scale investments in the fermentation plants. The other was the additional revenues from innovative or performance-enhanced products (The Economist, 2004a).

As biotechnological processes become cheaper, the economic contribution of industrial biotechnology will increase. That is why DuPont, in cooperation with Genencor, was trying to decrease by 25 per cent the production costs and by 20 per cent the investment costs of its polyester Sorona (manufactured at Decatur, Illinois), before launching its commercial production in 2004. Among the incentives for industrialists to invest in bioproducts or biofuels, which were still more expensive than their chemical equivalents, the OECD suggested to include the real cost of the environmental impact of industrial processes in market prices. The American Institute of Chemical Engineers proposed a total cost assessment method, which attempted to figure out in dollars all the risks posed on the environment, as well as on health and safety. Such a method and others like the 'green index' should entice an increasing number of enterprises to seize the opportunities offered by the bio-industry not only for the environment but also for the consumers and shareholders (The Economist, 2003).

Burrill & Co., a San Francisco-based venture capital firm, has created a \$50 million fund to invest in early-stage start-ups focused on biomaterials and bioprocessing. In the USA, representatives from different governmental bodies, industry, agriculture and academia worked together on a project called 'Vision 2020' with a view to boosting industrial biotechnology over the next decades backed by low feedstock prices and the support of the agricultural

community in the USA. The Farm Bill, passed in May 2002 by the US House of Representatives, authorized \$5 million in 2002 and \$14 million a year from 2003 through 2007 to fund biomass research and award grants to build 'biorefineries' – factories converting biomass into chemicals, fuels and energy. The Bill also requested the government to give preference to purchasing bio-based products (Reverchon, 2002).

Meanwhile, the European Bioindustries Association (EuropaBio) has proposed to the European Commission to form a strategic alliance to design a so-called Technology Platform. In order to stimulate global companies to invest in industrial biotechnology and in Europe, bridging incentives, more effective regulatory processes and low-cost feedstock were key factors.

Another switch that may prove crucial to watch is the move from ethanol to biotechnologically produced hydrogen. The driving power would not come from internal combustion engines but from fuel cells using hydrogen. In this regard, genomics may be the answer. Using the money Celera Genomics had raised, Craig Venter, who designed the whole-genome shotgun sequencing for both the human and microbial genomes, set up the Institute for Biological Energy Alternatives (Rockville, Maryland), with a view to studying microorganisms that produce hydrogen. One example is that of *Carboxydotherrmus*, discovered in a hydrothermal vent (deep-sea volcanic spring) off the coast of Russia, which draws its energy from reacting carbon monoxide with water, and produces hydrogen as a waste product. Another of Craig Venter's projects was to create a bacterium with a completely synthetic genome, leaving out the genes for carbohydrate synthesis that normally use hydrogen ions, so that the bacterium could devote all its energy pathways to producing hydrogen. Such unicellular micro-organism would not be able to live outside the laboratory because it would lack the biochemical mechanisms to survive there.

Discovering new materials for the industry while drawing on natural resources also speaks well for the prospects of industrial biotechnology. For instance, Nexia Biotechnologies, based in Quebec, is using technology similar to that of GTC Biotherapeutics to manufacture spider silk in goat's milk. Spider silk has been produced for the past 400 million years and has been adapted to a wide range of uses. This is an opportunity human beings could seize and use biotechnology-derived spider silk in many applications, from surgery to the design of new textiles.

6 Biomining and bleaching of ores

Chile is the world's first-biggest producer of copper. While in 1990 the production target had been fixed at 2.5 million tons for the year 2000, this figure was superseded in 1995, and the production exceeded 5 million tons in the late 1990s (*Bioplanet*, no. 6, July-August 2000, p.22). It has been exploiting copper since the 19th century with the help of local and foreign entrepreneurs, mainly from the USA. In 1971, copper mines were nationalized during the brief socialist episode of Chile's president Salvador Allende. Since then and despite the liberal economic policies conducted under the dictatorship of Augusto Pinochet and thereafter since 1990 when Chile returned to democracy, there has been no privatization in the mining sector with 80 per cent of the mines still presently owned by the state (Lazare, 2004). Because of the importance of copper mining and production in Chile's economy, the country has often been nicknamed the 'Saudi Arabia of copper'. Copper's physico-chemical properties and multiple uses, as well as a strong demand for the product from Asia (mainly China) have resulted in a high rise of international prices (London's stock exchange) during the first half of 2004 (Lazare, 2004).

Codelco, the National Copper Corporation, created in 1976, controls 20 per cent of the world's copper reserves and in the late 1990s produced around 2 million tons of the metal, accounting for some 13 per cent of world production. Copper represented 40 per cent of Chilean exports and one-third of Chile's earnings. According to its director-general, Codelco is planning to raise its annual production up to 3 million tons in 2012 (Lazare, 2004).

To illustrate the fast development of copper mining and production in Chile, one could cite the small city of El Salvador, located at an altitude of 2,600 metres and 1,000 km north-west of the capital Santiago; it was created during the 1950s with foreign partners in the middle of a desert area. Nowadays, out of its 10,000 inhabitants, 2,000 are working in copper mining and production. Codelco bears the expenses of housing costs of its workers as well as of the education of their worker's children. Water recycled from the copper mills is being used for producing vegetables, fruit and flowers, grown in greenhouses, as well as for feeding small livestock (Lazare, 2004).

The ore extracted from El Salvador's mines and containing about 2 per cent of copper is treated in a village in the same region, Llanta, so as to obtain a concentrate with 40 per cent of copper. This concentrate is thereafter treated in the furnaces of Potrerillos (99.5 per cent of copper) and then catalyzed to produce plates of pure copper (99.9 per cent). El Salvador is one of the five main divisions of Codelco. Its annual production only reached 85,000 tons of copper in 2003, but there are many prospects for extension. For instance, El Salvador could follow the model of Chuquicamata – the biggest open-air copper mine in the world, with an annual production of 600,000 tons – which was created at the beginning of the 20th century, partly with funding from US billionaires, including the Guggenheim. Chuquicamata extends over a huge basin 5-km long, 3-km wide and more than 850 m deep. The copper ore is extracted in the middle of the basin and transported in huge trucks to the surface to be treated. Every day, at 5

pm, a new explosion renews the stock of raw material to be processed (Lazare, 2004).

At El Teniente, south of El Salvador and another division of Codelco, a gigantic subterranean mine is being dug from the top of a mountain down to the surface level. Explosions are carried out in the heights; rocks are being ground downward, and loaded on a train at surface level. Codelco is not excluding any innovative solution to better exploit the 'national treasure' (Lazare, 2004).

In its first foray into the world of international corporate takeovers, Codelco and the Canadian company Noranda announced by mid-August 2000 a \$1.76-billion bid for Rio Algom, representing a 'major breakthrough' in Codelco's efforts to expand. But the joint bid was quickly topped by Billiton of the United Kingdom, with a \$2-billion agreed offer. Noranda and Codelco had to rethink their strategy, maybe with a new bid. Codelco's expansion plans were boosted in 2000 by a recovery in copper prices from a 12-year low in May 1999, from 60 cents to about 85 cents a pound. It also benefited from a cost-cutting programme forced on it by low copper price and cash costs were down 7 per cent to 41.1 cents a pound in the first half of 2000 (*Bioplanet*, no. 6, July-August 2000, p.22).

Codelco then partnered with Phelps Dodge of the USA, the world's second-biggest copper producer, to build the El Abra mine in Chile as well as prospecting alliance with the Mexican Grupo Penoles, the world's third-biggest producer. It also had a joint venture with Billiton, called Alliance Copper, which pioneered the commercial extraction of copper from low-grade ore using bioleaching. While the Chilean government ruled out selling Codelco, which gave up 10 per cent of its annual revenues to fund military expenses, the debate about privatization was continuing, particularly as large-scale utility sell-offs initiated in the late 1980s were nearing completion. The company nevertheless, seemed to be steadily moving toward a private-sector model (*Bioplanet*, no. 6, July-August 2000, p.22).

Bioleaching had been used only for the recovery of copper from wastes until the mid 1980's. The process was upgraded thanks to multidisciplinary and multi-institutional research efforts by several universities, research institutes and the productive sector, with the support of the Chilean government, the United Nations Development Programme (UNDP) and the United Nations Industrial Development Organization (UNIDO). In 2000, there were over five mines using bacterial bioleaching, and there were several projects to expand the use of this technology in the future, so as to raise its contribution to national copper production over 8 per cent to 10 per cent. The 5 million tons of copper produced in the late 1990s were the result of processing 400 million tons of ore, including 40 million tons treated via bioleaching (*Bioplanet*, no. 6, July-August 2000, p.22).

Back in Chile, the country had been exploring ways and means for applying bioleaching to gold production. Following the duplication of gold production in the country during the 1990s and the prospects for a 50 per cent increase in production during the three-year period 2000-

2002, the position of Chile is being fostered in this area (*Bioplanet*, no. 6, July-August 2000, p.22).

In July 2001, Chile's ministry of economy, the National Committee for Science and Technology Research (CONICYT) and the National Development Corporation (CORFO), in the presence of Chile's president, R. Lagos, entrusted the company BioSigma S.A. with pursuing a leadership in biotechnology applied to copper mining. This attracted a Japanese corporation, Nippon Mining & Metals Co. Ltd., to join BioSigma S.A., to establish leadership in biotechnologies involved in copper mining and in about one year the new company was created. Codelco's contribution was 66.6 per cent of the company's equity, while that of Nippon Mining & Metals Co. Ltd. amounted to one-third of equity. The Japanese corporation's contribution was not only financial, but it also opened the doors of for advanced technology and industry transfers among the countries involved.

BioSigma S.A. was the first example of an alliance between two major mining groups and a government which allocates competitive funds to attract research groups from Chilean and foreign universities to help industry transform basic knowledge into innovative products and services. Consequently, BioSigma S.A. elaborated a plan of technological development, which included the identification of specific microorganisms, technologies for the production of biomass of these microorganisms as well as the identification of specific genes encoding proteins that facilitate the bioleaching of copper sulfated ores. It was estimated that a minimum of one thousand genes would be identified in every microorganism. The expected outcome of the plan included technologies aimed at improving the current biomining processes, as well as more advanced solutions relating to the cloning or designing bacteria that increase bioleaching efficiency. BioSigma S.A.'s business plan set up short, medium and long-term tasks, and it was foreseen that BioSigma S.A. would recoup earnings from technology sale in 2005.

A major challenge for copper mining in Chile is the high concentration of arsenium and chloride in copper ores as well as the necessity to keep the mining wastes on the site and not to remove and dump them near urban settlements, e.g. around the city of Calama in the extreme north of the country. It was therefore necessary to design a safer and cheaper bioleaching process by arsenium-resistant and chloride-tolerant microorganisms, to be applied not only to new mining sites, but also to mining wastes from which the maximum amount of residual copper would be extracted.

Nippon Mining & Metals Co. Ltd., which is also a shareholder of several copper mines in Chile, including the well-known huge mine named La Escondida, has built a \$150-million pilot plant, in association with Codelco, to test the applicability of the bioleaching process named BioCop and developed in South Africa by the BHP consortium; in this pilot plant, tests included the use of chloride-tolerant hyperthermophilic micro-organisms.

The University of Antofagasta which has set up a Centre of Biotechnology and Molecular Biology dealing with

industrial and environmental biotechnology, has signed a biomining programme research contract with BioSigma S.A. Codelco, Nippon Mining and CONICYT have contributed \$2 million, \$2 million and \$1 million, respectively, to the biomining programme, which is part of CONICYT's Genomics Chile – the national genomics programme devoted to mining on the one hand and to agriculture and food on the other. The allocated budget allowed the building of BioSigma S.A.'s new reference laboratory in Santiago, the maintenance of the company's head office also in Santiago and the funding of four research projects – three Chilean and one Japanese. Three of the four projects were funded for the three-year period 2004-2006, and the fourth one was an innovative, exploratory research project to be carried out by the University of Antofagasta. The latter aimed at developing innovative bioremediation treatment of toxic mine effluents, i.e. transforming toxic metallic compounds into harmless substances. The three other research projects focused on sequencing the genomes of bioleaching microbes, cloning them to improve the bioleaching process, and on bioinformatics. See also Sasson (2000, pp.593-597).

7 Environmental biotechnology: bioremediation

Bioremediation is a key area of 'white' biotechnology, because the elimination of a wide range of pollutants from water and soils is an absolute requirement for sustainable development. There are numerous processes of cleaning water, industrial effluents and solid wastes, using microorganisms aerobically and anaerobically. Some of them are quite sophisticated, while others are simple and adapted to the conditions of developing countries. For instance, using microalgae (and in particular blue-green algae or cyanobacteria) in ponds to eliminate nitrogen and phosphorous, after organic matter has been degraded by bacteria, leads to water that can be recycled for irrigating non-food crops (e.g. cotton) or for industrial purposes; in addition, microalgal biomass can be used as feed.

7.1 Removal of toxic metals

Mercury is a highly toxic metal which, once released into water, accumulates in the food chain, damaging fish, shrimps and poisoning people who eat them. The infamous Minamata accident (called after a town on the Japanese island of Kyushu where the inhabitants suffered the toxic effects of fish poisoned by mercury-rich industrial effluents) is an example of the devastating effects of mercury on the central nervous system.

Existing techniques of mercury removal, such as precipitation or ion exchange, are expensive and not sufficiently efficient, as small but significant amounts of mercury still remain in the water. Researchers discovered that many bacteria had developed high tolerance to heavy metals, which related to the binding of these metals to proteins, e.g. metallo-thionein that binds mercury. As naturally thriving mercury-tolerant bacteria are rare and cannot be grown easily in culture, researchers at Cornell University, Ithaca, New York, inserted the metallo-thionein gene into *Escherichia coli*. A sufficiently large number of genetically engineered bacteria could thus treat mercury-polluted water inside a bioreactor. The efficiency of the procedure was high, as mercury was removed from polluted water down to a few nanograms per litre. Once the bacteria died, they were incinerated to recuperate the accumulated pure mercury (European Commission, 2002).

Mercury emissions were predicted to increase by 30 per cent throughout Europe between 1990 and 2010. The European Commission funded a demonstration project to show the feasibility and profitability of the microbial remediation technology under real time conditions. A plant was set up at Usti-nad-Labem in the Czech Republic, and has been operating since July 2000 (European Commission, 2002).

While phytoremediation is bioremediation based on the use of micro- and macro-algae, phytoremediation, relies on higher plants to clean water and soils from heavy metals and other pollutants, or to recolonize former mining areas (e.g. in South Africa, Australia, USA, Canada, France, etc.). For instance, heavy metals in industrial effluents can be concentrated in aquatic plants (e.g. the Azolla fern and water lentils – *Lemna* spp.) and thereafter recovered.

In 2003 in Western Europe, there were about 1.4 million

sites discovered to be polluted. Current remediation techniques are chemical or physico-chemical extraction techniques; they are costly and destroy soil structure. Phytoremediation techniques use "hyper-accumulating plants" which can store 10 to 500 more pollutants in their leaves and stems, which are thereafter harvested, incinerated, and metals recovered from ashes and reused in metallurgy.

The survey of hyper-accumulating plants started by the early 1990s. They are often small plants, such as *Alyssum murale*, which grows on metamorphic rocks, *Brassica juncea*, the Indian mustard, which extracts lead, or *Thlaspi*, which accumulates zinc and nickel. About 400 species have been identified, including 300 that accumulate only nickel. An endemic tree in New Caledonia, *Sebertia acuminata*, contains up to 20 per cent of nickel in its sap and is coloured in green (nickel is generally toxic to plants at a concentration of 0.005 per cent). In the Democratic Republic of Congo, the number of plants accumulating copper and cobalt is highest: 24 and 26 species, respectively. The accumulation efficiency is not generally very high. For some metals like silver, mercury and arsenium, there are yet no plants known to accumulate them. However, in 2000, the team of Lena Ma of the University of Florida, Gainesville, identified a fern, *Pteris vittata*, which tolerates and accumulates arsenium, while conserving a very rapid growth and a high biomass. Edenspace, a company from Virginia specialized in phytoremediation, acquired the rights to commercialize the fern (now called edenfern™) by signing an exclusive license agreement in 2000 with the University of Florida which patented the use of the fern in phytoremediation. In the US, seven or eight similar companies in phytoremediation were already in existence in 2002, where the value of the potential market for phytoremediation was estimated at \$100 million (Tastemain, 2004).

7.2 Bioremediation and genetic engineering

The molecular basis of heavy metal accumulation is being studied with a view to transferring the relevant genes to plant species having a wider geographic and ecological distribution. Transgenesis applied to phytoremediation is certainly incipient. Its application on a large scale is confronted with the evaluation of risks relating to the transfer of the bacterial transgenes to plants consumed by herbivorous animals that might acquire the property of hyper-accumulating toxic metals or compounds. Genetic transformation of the microorganisms involved in bioremediation could enhance the process through the introduction of genes controlling specific degradation pathways; it also aims at degrading recalcitrant compounds such as pesticides and other xenosubstances.

A team of US researchers led by Richard Meagher of the University of Georgia, Athens, were able to introduce into the genome of *Arabidopsis thaliana*, two foreign genes from *Escherichia coli* for the synthesis of two enzymes: one which catalyzes the transformation of arsenate into arsenite, the other which induces the formation of a complex with arsenite, that is retained in the leaves. These remarkable results led to transgenic *Arabidopsis* plants which could

accumulate three to four times more arsenium than normal plants and could become very effective depolluting agents. The same research team is well known for their “transgenic” work particularly in the transfer to plants of bacterial genes, coding for the conversion of toxic mercury into its less dangerous volatile form (Tastemain, 2002). In Cambridge, United Kingdom, Neil Bruce and his team are transforming tobacco plants with bacterial genes that control the synthesis of an enzyme detoxifying TNT (trinitrotoluene). Other researchers are studying the degradation of trichloroethylene by transgenic plants.

8 Illustrative examples

8.1 Thailand

Thailand provides a good illustration of the endeavours made by developing countries towards strengthening activities in environmental biotechnology and related areas. Through its Biochemical Engineering and Pilot Plant Research and Development Unit (BEC), set up in 1986 as a cooperative action between the National Centre for Genetic Engineering and Biotechnology (BIOTEC) and King Mongkut's University of Technology Thonburi (KMUTT). It has become one of a number of independent satellite research units of BIOTEC and this set-up enabled the researchers to work with less bureaucratic constraints.

During its second phase (1995-1999), BIOTEC became an autonomous organization and a large number of their researchers trained overseas and returned to Thailand to work with BIOTEC's satellite units. Also during this period, BIOTEC assigned university staff to administer the units. BEC's first five-year plan was implemented during this second phase, with emphasis on the collaboration between researchers from BIOTEC and KMUTT; this approach to shared resources proved a successful innovation for implementing research programmes in Thailand, producing a win-win situation.

The third phase (2000-2004) was implemented in four major specific areas: algal biotechnology, sensor technology, microbial bioprocess development, and waste utilization and management. The scope of BEC covers high quality research and development (R&D), technical services, technology transfer and consultancy, in order to support and strengthen the small and medium-sized companies' competitive position. In cooperation with the Industrial Park Center, Pilot Plant Development and Training Institute (PDTI) and School of Bioresources and Technology at KMUTT, BEC carries out its research work while being attentive to the demand pull (i.e. technology needs from industry) and the supply push (i.e. the innovation/knowledge provided by the research institutions).

BEC's funding comes from various sources: KMUTT, BIOTEC, research grants, contract research, technical services and consultancy, training, pilot-plant design, incubator hiring and analytical services. In the 2003 fiscal year, total input from KMUTT, BIOTEC and others were approximately 25.0, 17.5, and 20.6 million baht, respectively. In the area of waste utilization and management, research projects included:

- development of a kinetic model for anaerobic degradation;
- development of a hybrid reactor combining an upflow sludge bed and a fixed bed in treating wastes and producing biogas;
- population dynamics study of bacterial communities in an anaerobic hybrid reactor;
- benchmarking of water utilization in tapioca (cassava) starch industry.

High-rate anaerobic fixed film technology (HR-AFF) for agro-industrial wastewater treatment and energy recovery

has been developed up to the design and operation of an industrial-scale pilot plant. The advantage of HR-AFF compared with conventional systems lies in the fact that the bioreactor can handle large quantities of wastes per unit volume and produce biogas at a much faster rate. As a consequence, both the capital investment and operating costs of the process are lowered, resulting in a more economically viable system. The HR-AFF bioreactor can be used with low and high-strength organic wastewater and has been proven effective for several agro-industries, such as a rice-starch, tapioca-starch factories, and vegetable and fruit-canning plants. With respect to technical performance, the HR-AFF bioreactor can handle an organic loading rate at 2-10 kg COD/m³/day. The biogas yield was 0.4-0.5 m³/kg of COD removed which contained 60 per cent to 70 per cent methane. The efficiency of COD removal was 70 per cent-80 per cent. The HR-AFF system applied to the treatment of tapioca-starch waste-water could produce 25,000 m³ biogas/day, which could meet all the energy needs of the factory, normally met by 9,000 litres of fuel, and the rest of biogas could be used to generate electricity for sale back to the power grid of about 4,680 kWh/day.

Another achievement of BEC was the water and energy audit of tapioca-starch factories aimed at establishing a data-base and benchmarking of tapioca-starch production in Thailand for performance and efficiency improvement, and enhancement of trading potential in the global market. Five tapioca-starch factories were initially audited and the data obtained included: type of production, production capacity, and duration of operation, water and energy consumption. A second phase of the project targeted 20 factories.

The technology-transfer services provided by BEC to the industry include the following:

- design, construction and operation of an industrial-scale anaerobic fixed-bed reactor for waste-water treatment and biogas production for a rice-starch factory belonging to Bangkok Interfood Co. Ltd.;
- start-up anaerobic waste-water treatment plant and training for monitoring the system for a soft-drink manufacturer/bottler (Green Spot Company) since the company was able to run their waste-water treatment plant at full capacity with a high organic removal efficiency of 70 per cent-80 per cent; in addition, the cost of electricity to run the plant was considerably reduced;
- pilot-plant fermentation services applied to wastewater treatment in the shrimp-farming industry (Grandness Integration Co. Ltd., Allvet Co. Ltd. And T.C. Union Co. Ltd.); and
- contract research with Trangsure Co. Ltd. (frozen seafood and seafood canning factory) to improve the anaerobic sludge digester for treating excess sludge from their activated sludge systems and grease sludge from the dissolved air floatation unit.

Among the other achievements of BEC related to algal biotechnology and microbial fermentations, is the cultivation of *Spirulina*, which was developed from local strain selection first thru pilot studies and later, for

industrial-scale cultivation. The research involved the use of tapioca-starch wastewater as substrate for *Spirulina* cultivation in order to reduce production costs; the harvested algal slurry, once sun dried, contained 55 per cent protein and 7 per cent moisture. The operating costs for a plant covering a total production area (raceway-type ponds equipped with paddle wheels) of 1.5 hectares with a productivity of 40 tons of biomass per annum was estimated at between \$6,000 and \$7,000 per ton. The technology was transferred to the private sector where dried *Spirulina* is produced as high-protein feed for shrimps and fish. There seems to be good market opportunities for *Spirulina* biomass as food supplement for direct human consumption, in addition to its use as a feed supplement for animals; consequently the improvement of mass cultivation of the microalgae and its cost-effectiveness are important areas of research and development in Thailand and South-East Asia.

BEC has also done research on yeast. Local strains of baker's yeast and *Bacillus subtilis* with high yield have been selected and cultivated in a wide range of bioreactors from 5 litres up to 1,500 litres. Using locally-designed pilot-plant fermentation facilities, BEC has allowed potential private-sector clients to carry out small-scale production runs for feasibility, testing, cost predictions or optimization of processing steps, and production of prototype products for marketing tests. These projects with private sector clients included: a pilot-scale production of pressed yeast for Bioman Co. Ltd.; a probiotics market trial for *Bacillus subtilis* for use in the animal feed industry for Grand Siam Co. Ltd.; and cooperative research has been undertaken with Biowealth Co. Ltd. to conduct fungal fermentation for enzyme production applied to probiotics in the animal feed industry.

8.2 Australia

In Australia, in 2004, there were more than 60 Cooperative Research Centres (CRCs), which bring together researchers, and research users in industry, universities and government to carry out collaborative research in new, cross disciplinary areas. The CRCs that were working in fields as diverse as agriculture, mining and information technology, received about 25 per cent of their funding from the Australian government. Previously, on 30 October 2003 at the University of New South Wales, the Environmental Biotechnology Cooperative Research Centre (EBCRC) opened with research nodes in Brisbane, Perth and Adelaide, as well as two university sites in Sydney. It was a joint venture between industry, universities and government bodies. It will receive about A\$19-million government funding spread over seven years. Cash and 'in-kind' contributions from the joint venture participants and other sources will bring the centre's total budget over its programmed life to about A\$60 million.

Companies and research organizations that join EBCRC are required to make annual cash contributions and may also make 'in-kind' contributions in the form of facilities and staff. Experience has shown that the return on investment to industry members of CRC can be more than six times their investment in the CRC. Industries that would benefit from membership include aquaculture, brewing, chemicals,

food or paper processing, remediation, and oil refining and processing. The company will hold intellectual property arising from the EBCRC's research programme. Research results will be commercialized through spin-off companies, or participants in the EBCRC taking licenses for the new technologies, or direct third-party licenses.

Research projects have been selected to meet the following criteria: low to medium technical risk, time and cost constraints, and having significant potential return-on-investment. Five research projects or 'themes' have been approved to go ahead and two more have been identified for further funding namely: bioproducts from purple photosynthetic bacteria using wastes; rapid pathogen-detection strategies for the environment; novel biofilm control strategies; novel coatings for biofilm control and bioremediation; on-line process control and sludge population optimization for efficient nitrogen and phosphorus removal in high-strength wastes.

The overall goal of the EBCRC's work is to convert the by-products of industrial processes, currently regarded as waste, into high-value materials. For instance, meatworks (and the herders) obtain almost all their revenue from just half of each beast processed – the meat. Biotechnology could convert the remaining parts into biopolymers that could become biodegradable plastics or valuable chemicals. Breweries and food processors also produce wastes containing materials suitable for biotransformation. Outcomes of research projects will include:

- new fast, low-energy bioprocesses producing high-value products;
 - chemical precursors for manufacturing, e.g. esters, polyhydric alcohols (glycols);
 - new biosensors and control systems;
 - purification and separation processes for chemicals; and
 - reduced greenhouse gas emissions.
- The EBCRC also offers scholarships to students to undertake higher degrees and to develop the necessary research expertise across the full range of the centre's research. Students are provided with opportunities for work experience during their studies. Post-doctoral fellowships are also offered to attract skilled researchers, especially expatriates.

Through its research and training programme, the EBCRC aims at deeply changing the way in which Australia deals with waste and at showing how biotechnology can contribute to sustainability – whether in biofilms, bioproducts, biodecontamination, biofuels, or biotransformation, or just enhanced bioprocesses using the natural ecosystems.

9 Metagenomics

Craig Venter, the US biologist known for leading the private endeavour to sequence the human genome, is attempting to catalogue the microflora of oceanic environments. Onboard the *Sorcerer II*, it has already sailed along the east coast of North America, through the Panama Canal and around the Galapagos Islands. Every 200 miles, onboard researchers haul up 200 litres of seawater and filter it for microbes. Samples are frozen and sent to the USA, where biologists at the Institute for Biological Energy Alternatives in Rockville, Maryland, will try to sequence the DNA of every organism in each sample (Whitfield, 2004).

The expedition has the highest profile among many other similar projects under way around the world, in environments ranging from geographical (abandoned mines) to physiological (the human gut.) After sequencing the DNA of single species (there are complete sequences for more than 300 organisms), researchers are trying to sequence the DNA of living species in defined environments – a science called environmental genomics, or metagenomics. Through better understanding of how myriads of bacteria combine and interact to influence the ocean, we shall be better able to monitor the conditions of these environments and possibly manipulate them to our advantage. It may even be possible to find micro-organisms that can produce drugs or act as energy sources (Whitfield, 2004).

Science is now able to grow 3,000 species of bacteria on artificial culture media, but the vast majority – probably more than 99 per cent – resist such attempts. It seems impossible to approach such a huge microbial diversity with conventional techniques. Metagenomics can access this information. The principle is simple: select an environment; scoop or scrape up a sample for it; and sequence the genes in that sample. Lab researchers can look for diagnostic DNA sequences to count the number of species in a sample; they can put DNA fragments into bacteria that can be grown in the laboratory, and screen them for properties such as antibiotic production; or they can simply pull out sequences at random to give a picture of the number and type of genes in that environment. In practice, however, there is too much diversity in most environments to sequence every gene of every species (Whitfield, 2004).

Craig Venter's team collected a sample of 1,500 litres of water from the Sargasso Sea, in the Atlantic for their global study. They chose the Sargasso Sea because it was presumed to be poor in nutrients, and was thought to be one of the ocean's less diverse regions. But to their surprise, they found 1,800 species, including 150 new to science, and 1.2 million new genes. Environments richer in nutrients, such as the soil, will probably be even more diverse still. The pilot study in the Sargasso Sea cost \$2 million; C. Venter estimates that a more complete scan would currently cost five times that. The US Department of Energy funded the Sargasso Sea project (Whitfield, 2004). Thorough surveys of such places will have to wait for expected falls in the cost of sequencing.

Metagenomics could help us understand environmental problems such as carbon dioxide take up by bacteria to reduce its concentration, the breakdown of pollutants by

micro-organisms, It could also help to detect environmental damage, because once we know what it is that is out there, it is a step to be able to determine any changes to the environment (Whitfield, 2004).

Biotechnology companies such as Diversa, based in San Diego, are also working to mine the data for useful genes. A new antibiotic, turbomycin, was recently discovered during a metagenomic scan of soil by a team led by Jo Handelsman at the University of Wisconsin, Madison. For the time being, C. Venter's main objective is to gather information: 'the main purpose is cataloguing what is here and the diversity is higher than most people imagined; we are spending billions looking for life in Mars, but we do not have the slightest idea of what life there is on this planet' (Whitfield, 2004).

10 Social acceptance

The potential of 'white' biotechnology is conducive to the tie-ups and linkages among government, industry and academe to work within a sustainable development framework. By providing alternatives such as new materials and fuels that are not derived from petrochemical processes, by improving and enhancing the bioremediation of water, soils and ecosystems at large, by trying to use less fossil-fuel energy, 'white' biotechnology will enjoy a positive social acceptance, even among environmentalists, if it continues on this path.

However, a crucial factor in social acceptance particularly by environmentalists is the conclusive impact on biodiversity of releasing into the environment genetically-modified organisms used in the processes of 'white' biotechnology, including bioremediation; and this is still largely unknown. Industrialists use "white biotechnology" mostly in confined environments, such as their factories, bioreactors and greenhouses, while applying strict biosafety regulations. Until then, widespread social acceptance may have to wait.

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